

10/517052

Figure 1A

80 TGATAAAAAT TGATAAAAAT TGATAAAAAT TGATAAAAAT TGATAAAAAT	160 TAAATAATCA TAAATAATCA TAAATAATCA TAAATAATCA	240 GAAAAACATT GAAAAACATT GAAAAACATT GAAAAACATT	320 GCTCGAAGCG GCTCGAAGCG GCTCGAAGCG GCTCGAAGCG	400 GGTGCAAAGC GGTGCAAAGC GGTGCAAAGC GGTGCAAAGC
CTACAAAAT CTACAAAAAT AAAT CTACAAAAAT ctacaaAAAT	GACAACACAA GACAACACAA GACAACACAA GACAACACAA	CAATACAATG CAATACAATG CAATACAATG CAATACAATG CAATACAATG	TTGGTGATGA TTGGTGATGA TTGGTGATGA TTGGTGATGA	AAGTGTTGGA AAGTGTTGGA AAGTGTTGGA AAGTGTTGGA
GAGAAAT GAGAA-T GAG GAGTGAAAAT GAGaa.t	ATTCGCTTTA ATTCGCTTTA ATTCGCTTTA ATTCGCTTTA	GATAATCATA GATAATCATA GATAATCATA GATAATCATA GATAATCATA	CGGGCTGACA CGGGCTGACA CGGGCTGACA CGGGCTGACA	CATAATAGAC CATAATAGAC CATAATAGAC CATAATAGAC
AATTCGGCAC AATTCGGCAC AATTCGGCAC AATTCGGCAC	TGGCTACATG TGGCTACATG TGGCTACATG TGGCTACATG	TGTAAAAAA TGTAAAAAAA TGTAAAAAAA TGTAAAAAAA	TGCAGCTGCT TGCAGCTGCT TGCAGCTGCT TGCAGCTGCT	CAGCACATAA CAGCACATAA CAGCACATAA CAGCACATAA CAGCACATAA
GG GGGCTGCAGG GGGCTGCAGG	CTTGCATACA CTTGCATACA CTTGCATACA CTTGCATACA	GATAGAAAGT GATAGAAAGT GATAGAAAGT GATAGAAAGT	TTTTCGTGGG TTTTCGTGGG TTTTCGTGGG	CACGAATGTG CACGAATGTG CACGAATGTG CACGAATGTG CACGAATGTG
TGGATCCCC TGGATCCCCC	GAGTCATTGG GAGTCATTGG GAGTCATTGG GAGTCATTGG	GGTACTTTGA GGTACTTTGA GGTACTTTGA GGTACTTTGA	GCAGCGTTTG GCAGCGTTTG GCAGCGTTTG GCAGCGTTTG	GAGGGGCTTA GAGGGGCTTA GAGGGGCTTA GAGGGGCTTA
1 GCGGCCGCT CTAGAACTAG GGCGGCCGCT CTAGAACTAG	81 AAAAATAACAAAGC AAAAATAAA ATAACAAAGC AAAAATAAAA ATAACAAAGC AAAAATAAAA ATAACAAAGC	161 GCAATATATA AAGTACCTTC GCAATATATA AAGTACCTTC GCAATATATA AAGTACCTTC GCAATATATA AAGTACCTTC	ATTGTTCACC ATTGTTCACC ATTGTTCACC ATTGTTCACC	321 GCTCAATTTA ATTCAACAG GCTCAATTTA ATTCAACAG GCTCAATTTA ATTCAACAG GCTCAATTTA ATTCAACAAG
1 GCGGCCGCT GGCGGCCGCT	81 AAAAATAAA AAAAATAAAA AAAATAAAA AAAAATAAAA	161 GCAATATATA GCAATATATA GCAATATATA GCAATATATA	241 ATTTGTTAT ATTTGTTAT ATTTGTTAT ATTTGTTAT	321 GCTCAATTTA GCTCAATTTA GCTCAATTTA GCTCAATTTA
M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA



Figure 1B

480 TTGGAGGGG TTGGAGGGG TTGGGAGGGG	560 TGTCACCCAA TGTCACCCAA TGTCACCCAA TGTCACCCAA	640 AAGACAAGAC AAGACAAGAC AAGACAAGAC AAGACAAGAC	720 ATCATTCATA ATCATTCATA ATCATTCATA ATCATTCAT	800 TAGACATAAG TAGACATAAG TAGACATAAG TAGACATAAG
AACTGGCGGA AACTGGCGGA AACTGGCGGA AACTGGCGGA	TTCGTTGTGG TTCGTTGTGG TTCGTTGTGG TTCGTTGTGG	GTGATAAACA GTGATAAACA GTGATAAACA GTGATAAACA GTGATAAACA	TTGCAATGTG TTGCAATGTG TTGCAATGTG TTGCAATGTG	TATTAAGGCA ACGGACGCTA AACCAGGACA TATTAAGGCA ACGGACGCTA AACCAGGACA TATTAAGGCA ACGGACGCTA AACCAGGGCA TATTAAGGCA ACGGACGCTA AACCAGGACA
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CAAATGCGCG	ACAATGCTGC	GATATGGTCA	CACTTGTGGC	AAAGTAACCG AAGGTGGAAT
CAAATGCGCG	ACAATGCTGC	GATATGGTCA	CACTTGTGGC	AAAGTAACCG AAGGTGGAAT
CAAATGCGCG	ACAATGCTGC	GATATGGTCA	CACTTGTGGC	AAAGTAACCG AAGGTGGAAT
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AAGCATTAGC	TGTTCAGATG	CTTTAAGAAA	ATGTTGAGAT	
AAGCATTAGC	TGTTCAGATG	CTTCAAGAAA	ATGTTGAGAT	
AAGCATTAGC	TGTTCAGATG	CTTTAAGAAA	ATGTTGAGAT	
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401	481 AAATCTACGT AAATCTACGT AAATCTACGT AAATCTACGT	561	641	721
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TGATTGGGAA AAAAACCGAC		GATAAACCTT	AATTGATGGA	ACATTCACAT
M4cDNA	M4cDNA	M4cDNA	M4cDNA	M4cDNA
M6cDNA	M6cDNA	M6cDNA	M6cDNA	M6cDNA
M8cDNA	M8cDNA	M8cDNA	M8cDNA	M8cDNA
M15cDNA	M15cDNA	M15cDNA	M15cDNA	M15cDNA
Consensus	Consensus	Consensus	Consensus	Consensus

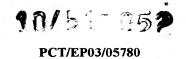


Figure 1C

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CTTAGTCATG CTTAGTCATG CTTAGTCATG CTTAGTCATG	TCACCAAAAA TCACCAAAAA TCACCAAAAA TCACCAAAAA	GGTTCGCAGA GGTTCGCAGA GGTTCGCAGA GGTTCGCAGA	TGGGGAACGT TGGGGAACGT TGGGGAACGT TGGGGAACGT	aatgaagaa aatgaagaaa aatgaagaaa aatgaagaaa
TCATTGCACA TCATTGCACA TCATTGCACA TCATTGCACA	AATTTAGCCA AATTTAGCCA AATTTAGCCA AATTTAGCCA	GCATTCAACA GCATTCAACA GCATTCAACA GCATTCAACA GCATTCAACA	CTACACCAGC CTACACCAGC CTACACCAGC CTACACCAGC	CGAATGACCC CGAATGACCC CCAATGACCC CGAATGACCC
TATGGATCGA TATGGATCGA TATGGATCGA TATGGATCGA	TCCAATTGCA TCCAATTGCA TCCAATTGCA TCCAATTGCA	TGTCACAGTA TGTCACAGTA TGTCACAGTC TGTCACAGTA	TTAACAATGA TTAACAATGA TTAACAATGA TTAACAATGA	TTCCATGCTC TTCCATGCTC TTCCATGCTC TTCCATGCTC
TCTTCAAAGA TCTTCAAAGA TCTTCGAAGA TCTTCAAAGA	CGTTACCALT CGTTACCALT CGTTACCALT CGTTACCALT	GTAGACGATA AAAAAATGCA GTAGACGATA AAAAAATGCA GTAGACGATA AAAAAATGCA GTAGACGATA AAAAAATGCA	TTCCAAGTTG TTCCAAGTTG TTCCAAGTTG TTCCAAGTTG	TCCTTAGCCA AGGCAACCGA TCCTTAGCCA AGGCAACCGA TCCTTAGCCA AGGCAACCGA TCCTTAGCCA AGGCAACCGA
TGTTGCTGGT TGTTGCTGGT TGTTGCTGGT TGTTGCTGGT	GTAGCACAGC GTAGCACAGC GTAGCACAGC GTAGCACAGC		ATTIGGATTT ATTIGGATTT ATTIGGATTT ATTIGGATTT ATTIGGATTT	
801 AGCGACGGAG ATGGTATTTG AGCGACGGAG ATGGTATTTG AGCGACGGAG ATGGTATTTG AGCGACGGAG ATGGTATTTG	GTCACGTTGG GTCACGTTGG GTCACGTTGG GTCACGTTGG	CAATTCACAT CAATTCACAT CAATTCACAT CAATTCACAT	CACGATGTCG CACGATGTCG CACGATGTCG CACGATGTCG	AATCCTACTA AATCCTACTA AATCCTACTA AATCCTACTA AATCCTACTA
801 AGCGACGGAG AGCGACGGAG AGCGACGGAG AGCGACGGAG	881 CCTTATTGAT CCTTATTGAT CCTTATTGAT CCTTATTGAT	961 TCGGAGCAGA TCGGAGCAGA TCGGAGCAGA TCGGAGCAGA	1041 CAAAGAATGC CAAAGAATGC CAAAGAATGC CAAAGAATGC	1121 TGGTAGTGCC AA TGGTAGTGCC AA TGGTAGTGCC AA
M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA Consensus

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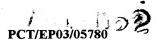


Figure 1D

TGGAGCTATA TGGAGCTATA TGGAGCTATA TGGAGCTATA	1360 GATCAGCAGT GATCAGCAGT GATCAGCAGT GATCAGCAGT	1440 ACTTTTTTT ACTTTTTATT ACTTTTTATT ACTTTTTATT	1520 ACATTGATAC GTGCATTACA AGACCATTCA ACATTGATAC GTGCATTACA AGACCATTCA ACATTGATAC GTGCATTACA AGACCAAAAA ACATTGATAC GTGCATTACA AGACCAAAAA ACATTGATAC GTGCATTACA AGACCAAAAA	
TGTTAGAAAA TGGAGCTATA TGTTAGAAAA TGGAGCTATA TGTTAGAAAA TGGAGCTATA TGTTAGAAAAA TGGAGCTATA	1360 TTTGATTCCA GCTGAACCAG GATCAGCAGT TTTGATTCCA GCTGAACCAG GATCAGCAGT TTTAATTCCA GCTGAACCAG GATCAGCAGT TTTGATTCCA GCTGAACCAG GATCAGCAGT	1440 CTTGTTAATA GTTATCACCC ACTTTTTTT CTTGTTAATA GTTATCACCC ACTTTTTATT CTTGTTAATA GTTATCACCC ACTTTTTATT CTTGTTAATA GTTATCACCC ACTTTTTATT CTTGTTAATA GTTATCACCC ACTTTTTATT	1520 ACATTGATAC GTGCATTACA AGACCATTCA ACATTGATAC GTGCATTACA AGACCAAAAA ACATTGATAC GTGCATTACA AGACCAAAAA ACATTGATAC GTGCATTACA AGACCAAAAA	
GAGAAAGACT GAGAAAGACT GAGAAAGACT GAGAAAGACT GAGAAAGACT			ACATTGATAC ACATTGATAC ACATTGATAC ACATTGATAC	
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1201 TGAGGGCTGA TGCACCACAT ACAGAGTCAA TGAAGTGGAA TGAGGGCTGA TGCACCACAT ACAGAGTCAA TGAAGTGGAA TGAGGGCTGA TGCACCACAT ACAGAGTCAA TGAAGTGGAA TGAGGGCTGA TGCACCACAT ACAGAGTCAA TGAAGTGGAA	CCCGCATCTA ACCCCGGAAC AAAAAAGCCA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA CCCGCATCTA ACCCCGGAAC AAAAAAGCCA	CAAATGCGTT CAAATGCGTT CAAATGCGTT CAAATGCGTT		
CO1 CGAGGCTGA TGCACCACAT ACAGAGTCAA CGAGGGCTGA TGCACCACAT ACAGAGTCAA CGAGGGCTGA TGCACCACAT ACAGAGTCAA CGAGGGCTGA TGCACCACAT ACAGAGTCAA CGAGGGCTGA TGCACCACAT ACAGAGTCAA	CCCGCATCTA CCCGCATCTA CCCGCATCTA CCCGCATCTA	TCTTCAACTC ACCAGTTGTG CTGGCACGCT	TTATACTTGT TTATACTTGT TTATACTTGT TTATACTTGT	
1201 TGAGGGCTGA TGCACCACAT ACAGAGTCAA	1281 ITTGTAGCAT CAGGGTGCGA ITTGTAGCAT CAGGGTGCGA ITTGTAGCAT CAGGGTGCGA ITTGTAGCAT CAGGGTGCGA	1361 TCTTCAACTC ACCAGTTGTG TCTTCAACTC ACCAGTTGTG TCTTCAACTC ACCAGTTGTG	1441 TTTATTGTTA TTAGCATTTT TTTATTGTTA TTAGCATTTT TTTATTGTTA TTAGCATTTT TTTATTGTTA TTAGCATTTT	
1201 TGAGGGCTGA TGAGGGCTGA TGAGGGCTGA TGAGGGCTGA	1281 TTTGTAGCAT TTTGTAGCAT TTTGTAGCAT TTTGTAGCAT	1361 TCTTCAACTC TCTTCAACTC TCTTCAACTC	1441 TTTATTGTTA TTTATTGTTA TTTATTGTTA TTTATTGTTA	
M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	M4cDNA M6cDNA M8cDNA M15cDNA Consensus	

tcAAcAtAtt ttgcta..a.aaaaaaa aaaaaaaa ---- AAAAAA AAAAAAAA TCAACATATT TTGCTAAAAааааааааа аааа ааааааааа ааааа M8cDNA M15cDNA M6cDNA Consensus

TCAACATATT TTGCTACTAT CACATGTTCA CGTTAAAAAA AAAAAAAAA

M4cDNA

Figure 2

cDNA and amino acid sequences of M4, M6, M15 (identical clones):

MEKHYFVILFTAAFVFVGAA gctcgggctgacattggtgatgagctcgaagcggctcaatttaattcaacaaggagggc A R A D I G D E L E A A Q F N S T R R G ttacacgaatgtgcagcacataacataatagacaagtgttggaggtgcaaagctgattgg LHECAAHNIIDKCWRCKADW gaaaaaaaccgacaagcattagccaaatgcgcgcaaggttttgcaaagggaacaactggc E K N R Q A L A K C A Q G F A K G T T G ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca G L G G E I Y V V T D C S D D N A A N P aagccagggacacttcgttgtggtgtcacccaagataaacctttgtggatcatctttaag K P G T L R C G V T Q D K P L W I I F K aaagatatggtcataaaacttaaacacgagcttgtgataaacaaagacaagacaattgat K D M V I K L K H E L V I N K D K T I D ggaagaggtgcaaatgttgagatcacttgtggcggtctcaccattcacaacgtttgcaat G R G A N V E I T C G G L T I H N V C N gtgatcattcataacattcacatacatgatattaaagtaaccgaaggtggaattattaag V I I H N I H I H D I K V T E G G I I K gcaacggacgctaaaccaggacatagacataagagcgacggagatggtatttgtgttgct A T D A K P G H R H K S D G D G I C V A ggttcttcaaagatatggatcgatcattgcacacttagtcatggtccagatggccttatt G S S K I W I D H C T L S H G P D G L I gatgtcacgttgggtagcacagccgttaccatttccaattgcaaatttagccatcaccaa D V T L G S T A V T I S N C K F S H H Q aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca K I L L G A D N S H V D D K K M H V T gtagcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga V A F N R F A E A C D Q R M P R C R F G tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggtagt gccaatcctactatccttagccaaggcaaccgattccatgctccgaatgacccaatgaag A N P T I L S Q G N R F H A P N D P M K aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtggaattggaga K N V L V R A D A P H T E S M K W N W R tctgagaaagacttgttagaaaatggagctatatttgtagcatcagggtgcgacccgcat S E K D L L E N G A I F V A S G C D P H ctaaccccggaacaaaaagccatttgattccagctgaaccaggatcagcagttcttcaa LTPEQKSHLIPAEPGSAVLQ ctcaccagttgtgctggcacgctcaaatgcgttcctggaaaaccttgttaa L T S C A G T L K C V P G K P C -

Figure 3

cDNA and amino acid sequence of clone M8:

MEKHYFVILFTAAFVFVGAA gctcgggctgacattggtgatgagctcgaagcggctcaatttaattcaacaaggagggc A R A D I G D E L E A A Q F N S T R R G ttacacgaatgtgcagcacataacataatagacaagtgttggaggtgcaaagctgattgg LHECAAHNIIDKCWRCKADW gaaaaaaaccgacaagcattagccaaatgcgcgcaaggttttgcaaagggaacaactggc EKNRQALAKCAQGFAKGTTG ggattgggaggggaaatctacgtggtgactgattgttcagatgacaatgctgcaaatcca G L G G E I Y V V T D C S D D N A A N P aagccagggacacttcgttgtggtgtcacccaagataaacctttgtggatcatcttcaag K P G T L R C G V T Q D K P L W I I F K aaagatatggtcataaaacttaaacacgagcttgtgataaacaaagacaagacaattgat K D M V I K L K H E L V I N K D K T I D ggaagaggtgcaaatgttgagatcacttgtggcggtctcaccattcacaacgtttgcaat GRGANVEITCGGLTIHNVCN gtgatcattcataacattcacatacatgatattaaagtaacggaaggtggaattattaag V I I H N I H I H D I K V T E G G I I K gcaacggacgctaaaccagggcatagacataagagcgacggagatggtatttgtgttgct A T D A K P G H R H K S D G D G I C V A ggttcttcgaagatatggatcgatcattgcacacttagtcatggtccagatggccttatt G S S K I W I D H C T L S H G P D G L I gatgtcacgttgggtagcacagccgttaccatttccaattgcaaatttagccatcaccaa D V T L G S T A V T I S N C K F S H H Q aaaattctattactcggagcagacaattcacatgtagacgataaaaaaatgcatgtcaca KILLLGADNSHVDDKKMHVT gtcgcattcaacaggttcgcagaagcatgtgatcaaagaatgccacgatgtcgatttgga V A F N R F A E A C D Q R M P R C R F G tttttccaagttgttaacaatgactacaccagctggggaacgtacgccattggtggtagc F F Q V V N N D Y T S W G T Y A I G G S gccaatcctactatccttagccaaggcaaccgattccatgctcccaatgacccaatgaag ANPTILSQGNRFHAPNDPMK aaaaatgtgttggtgagggctgatgcaccacatacagagtcaatgaagtggaattggaga K N V L V R A D A P H T E S M K W N W R tctgagaaagacttgttagaaaatggagctatatttgtagcatcagggtgcgacccgcat S E K D L L E N G A I F V A S G C D P H ctaaccccggaacaaaaagccatttaattccagctgaaccaggatcagcagttcttcaa LTPEQKSHLIPAEPGSAVLQ ctcaccagttgtgctggcacgctcaaatgcgttcctggaaaaccttgttaa L T S C A G T L K C V P G K P C -

Figure 4

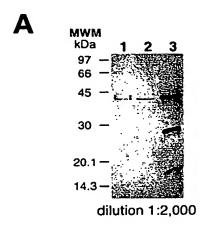
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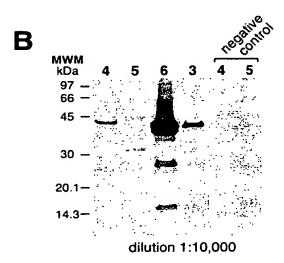
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GAA AAC CTG TAT TTT CAG GGC GG Glu Asn Leu Tyr Phe Gln Gly TTEV Protease Cleavage Site	Nco I Bam HI Eco RI	CAA AGG CCT ACG TO	CG ACG AGC er Thr Ser
Spe I Not I Nsp V TCA ACT AGT GCG GCC GCT TTC G Ser Thr Ser Ala Ala Ala Phe G	AA TCT AGA GCC TGC AGT	XhoI CTC GAG CAC CAC C Leu Glu His His H	AC CAC CAC is His His
CAC TGA GAT CCG GCT GCT AAC A	AA GCC CGA AAG GAA GCT	GAG TTG GCT GCT G	CC ACC GCT
*Non-unique sites			



Figure 5

Immunoblot with rabbit anti-Amb a 1 antibodies



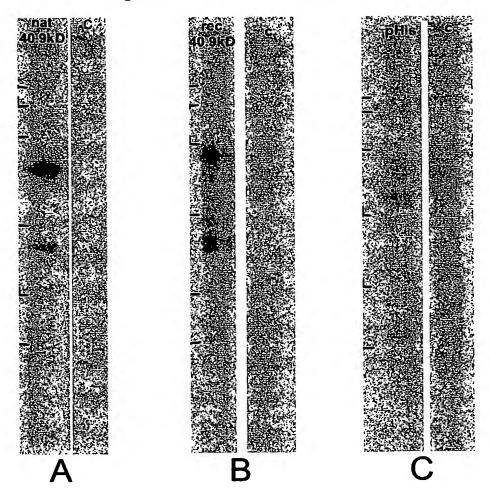


- 1 Mugwort pollen extract
- 2 Purified mugwort pollen allergen
- 3 Purified Amb a 1 from ragweed pollen (natural Amb a 1)
- 4 Recombinant mugwort allergen
- 5 Control bacterial lysate
- 6 Ragweed pollen extract

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Figure 6

IgE blot with NIH patient



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Figure 7



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